



Increased Levels of Interleukin-17A Exosomes in Psoriasis

Claire JACQUIN-PORRETAZ¹, Marine CORDONNIER², Charlée NARDIN^{1,3}, Laura BOULLEROT⁴, Gaetan CHANTELOUP², Valentin VAUTROT^{2,3}, Olivier ADOTEVI⁴, Carmen GARRIDO², Jessica GOBBO^{2,5#} and François AUBIN^{1,3#}

¹Department of Dermatology, University Hospital, Besançon, ²University of Bourgogne-Franche Comté, Inserm U1231, Dijon, ³EA3181 and ⁴UMR Inserm 1098, University of Bourgogne-Franche Comté, Besançon, and ⁵Department of Medical Oncology, Early Phase Units, Georges-François Leclerc Centre, Dijon, France

[#]These authors contributed equally.

Exosomes are involved in modulating the immune system and mediating communication between cells. The aim of this study was to investigate the involvement of exosomes in psoriasis. Exosomes from patients with psoriasis were analysed by nanoparticle tracking analysis and protein expression was analysed by western blotting. The concentration of HSP70 was determined by an enzyme-linked immunosorbent assay, and concentrations of interleukin (IL)-1 β , IL-2, IL-6, IL-10, IL-17A and tumour necrosis factor alpha (TNF- α) were determined by flow cytometry. Based on the severity of psoriasis, evaluated by body surface area ($\leq 10\%$ vs. $> 10\%$), 2 groups of patients were compared (49 with mild psoriasis and 71 with moderate-to-severe psoriasis). The number ($2.52 \times 10^{11} \pm 2.29 \times 10^{10}$ vs. $1.79 \times 10^{11} \pm 1.93 \times 10^{10}$, $p = 0.19$) and size (94.44 ± 22.00 nm vs. 96.87 ± 28.30 nm, $p = 0.72$) of exosomes and the concentration of HSP70 in the exosomes were not significantly different in the 2 groups of patients. IL-17A exosome levels were significantly higher in patients with moderate-to-severe psoriasis compared with those with mild psoriasis ($p = 0.02$). There were no significant differences in levels of TNF- α , IL-1, IL-2, IL-6 and IL-10. This study shows, for the first time, the presence of circulating exosomes in patients with psoriasis. These data confirm the involvement of circulating exosomes in psoriasis, in particular in moderate-to-severe psoriasis, through IL-17A-producing exosomes.

Key words: exosomes; psoriasis; IL-17.

Accepted Aug 22, 2019; E-published Aug 22, 2019

Acta Derm Venereol 2019; 99: 1143–1147.

Corr: François Aubin, Department of Dermatology, University Hospital, 3 Bd Fleming, FR-25030 Besançon, France. E-mail: francois.aubin@univ-comte.fr

Exosomes are naturally occurring small membrane-enclosed nanovesicles with a characteristic diameter of 30–120 nm, which are constitutively generated and released by various cells (1). Exosomes are shed from the surface of most cell types. They carry membrane components and contents from the cytoplasm of the cells from which they are released. Under normal cellular conditions, the release of exosomes accompanies normal cell growth and activation of some cellular functions, such as stimulation of T-cell growth *in vitro* and induction of anti-tumour immune responses depending on

SIGNIFICANCE

Exosomes are naturally occurring small membrane-enclosed nanovesicles that modulate the immune system and mediate communication between cells and the transport of cellular components. Although the field of exosome research in cancer progression is expanding, there are very few studies on the role of exosomes in immune-mediated inflammatory diseases. This study shows, for the first time, the presence of circulating exosomes in patients with psoriasis, in particular in those with moderate-to-severe psoriasis, through IL-17A-producing exosomes. Further larger studies are required, to evaluate the effect of systemic treatments on exosome production.

the specific cell type (2). These vesicles contain various DNA (3), miRNA (4) and proteins (5). They can enter the bloodstream and act as messengers between cells (1).

Although the field of exosome research in cancer progression is expanding, there are very few studies into the role of exosomes in immune-mediated inflammatory diseases (6, 7). Psoriasis is a chronic, autoimmune skin disease associated with a T-helper-17 response induced by the release of IL-23 from myeloid epidermal dendritic cells, leading to proliferation of keratinocytes, resulting in psoriatic plaque formation (8).

Although recent biologic agents targeting different pro-inflammatory cytokines have revolutionized the management of psoriasis, these agents do not address the causes of the abnormal immunoregulatory responses of the disease, because the aetiology is not yet completely understood. The 70-kDa heat shock proteins (HSP70) are known to be potent immunomodulators, and their anti-inflammatory activity has recently been investigated in a murine psoriasis-like model (9).

Since exosomes can modulate the immune system and mediate communication between cells and the transport of cellular components, such as cytokines and HSP70 (10), the aim of this study was to investigate their involvement in psoriasis.

PATIENTS AND METHODS

Patients

The study design was approved by the local research ethics committee and written informed consent was provided before

enrolment for patients with plaque psoriasis. This prospective monocentric study adhered to the principles of the Declaration of Helsinki and was conducted between November 2016 and December 2017 in the Department of Dermatology, University Hospital of Besançon, France. Demographic data were collected for each patient. Body surface area (BSA), Physician Global Assessment (PGA) and Dermatology Quality of Life Index (DLQI) were evaluated by the same investigator (FA) or by the patients themselves throughout blood sampling. Blood samples were collected in EDTA tubes in order to perform analysis on exosomes.

Exosome purification

Exosomes were isolated from psoriatic patients' blood samples as described previously (11). Samples were first centrifuged at 1,500 rpm for 15 min, then supernatant was retrieved and stored at -22°C . Samples were then centrifuged at 17,000 g for 10 min (4°C). Exosomes were then ultracentrifuged at 200,000 g for 60 min and the pellet containing exosomes was re-suspended in a suitable volume of either phosphate-buffered saline or radioimmunoprecipitation assay (RIPA) lysis buffer (ThermoFisher Scientific, Illkirch-Graffenstaden, France) for western blotting and cytokine analysis.

Characterization of purified exosomes

Exosomes derived from plasma of patients isolated by ultracentrifugation were evaluated for their size and concentration by nanoparticle tracking analysis using a NS300 Instrument (Nanosight, Amesbury, UK, Software 3.0) and protein expression was analysed by western blotting. Proteins were separated in 10% SDS-polyacrylamide gel and transferred to polyvinylidene difluoride (PVDF) membranes as described (15). Primary antibodies used were TSG101 (sc-7964, Santacruz, Heidelberg, Germany), Alix (NB100-65678, Novusbio, Lille, France), CD9 (ab92726, Abcam, Paris, France), Syntenin-1 (sc-100336, Santacruz), and HSP70 (ADI-SPA-810-F, EnzoLife, Villeurbanne, France). After incubation with appropriate secondary antibodies (1/20,000) coupled with horseradish peroxidase (Jackson ImmunoResearch Laboratories, Eli, UK) membranes were revealed with ECL (Amersham).

Analysis of HSP70 in exosomes

Concentration of HSP70 in psoriatic patient-derived exosomes was determined using an enzyme-linked immunosorbent assay (HSP70 high sensitivity kit, EnzoLife) according to the manufacturer's protocol. Proteins concentrations were determined according to standard curves.

Analysis of cytokines in the exosomes

The exosome concentrations of IL-1- β , IL-2, IL-6, IL-10, IL-17A and TNF- α were measured with the BD Cytometric Bead Array Flex Sets (BD Pharmingen, BD Biosciences, Le Pont de Claix, France), according to the manufacturer's instructions, and analysed on a FACS Canto II flow cytometer using BD FACS Diva and FCAP Array software (BD Biosciences).

Statistical analysis

Clinical and biological data were analysed with SAS[®] software (Grégy-sur-Yerres, France) and biostaTGV program (Institut Pierre Louis UMR S 1136, Paris, France). Quantitative results are expressed as means \pm standard deviations (SD) from at least 3 independent experiments. Statistical significance was determined using an unpaired, 2-tailed Student's *t*-test, or analysis of variance (ANOVA) ($p < 0.05$).

RESULTS

Demographic characteristics

A total of 81 patients (50 males, 31 females) with psoriasis, age range 14–82 years (mean 48 ± 16.3 years) were included. Seven patients (8.6%) had psoriatic arthritis. The mean body mass index (BMI) was 29.6 ± 6.7 kg/m² (median 29.5 kg/m²) and 23 patients (28.4%) had a BMI exceeding 30 kg/m² (Table I).

Psoriasis severity

Mean BSA was $23 \pm 19\%$ (0–90%, median 30%). Mean PGA score was 1.75 ± 1.36 (median PGA 2). Mean DLQI was 9.42 ± 8.22 . At the time of sampling, all patients received systemic treatment. Twenty-two patients (27%) were treated with methotrexate, 19 (24%) with adalimumab, 12 (15%) with secukinumab, 10 (12%) with ixekinumab, 9 (11%) with ustekinumab, 6 (7%) with apremilast, 2 with etanercept (2%) and 1 patient (1%) simultaneously received methotrexate and adalimumab.

Two groups of patients were compared depending on psoriasis severity evaluated by BSA (10): mild psoriasis (BSA $\leq 10\%$) and moderate-to-severe psoriasis (BSA $> 10\%$) Thus, 28 patients were included in the mild psoriasis group, with a mean DLQI of 2.2 ± 4.4 , and 53 patients were included in the moderate-to-severe group, with a mean DLQI of 14.3 ± 6.3 (median 14). BSA and DLQI were significantly different between patients with mild psoriasis and those with moderate-to-severe psoriasis. There were no statistically significant differences concerning psoriatic arthritis, BMI, sex or age (Table I).

Exosomes and psoriasis

To avoid any artefact related to the presence of other microvesicles potentially co-purified with exosomes, the expression of specific classical markers of exosomes, such as TGS101 and Alix, was validated in all samples. The mean number of exosomes was $2.21 \times 10^{11} \pm 2.16 \times 10^{10}$ and their mean size was 95.49 ± 24.71 nm. There was no significant difference, compared with a group of 10 control healthy patients (mean exosome number $1.73 \times 10^{11} \pm 5.36 \times 10^{10}$, $p = 0.15$). Because of the limited quantity of plasma in some patients, exosomes were

Table I. Patients' characteristics

	Mild psoriasis BSA $\leq 10\%$ (<i>n</i> = 49)	Moderate-to-severe psoriasis BSA > 10 (<i>n</i> = 71)	<i>p</i> -value
DLQI, mean \pm SD	2.2 \pm 4.4	14.3 \pm 6.3	<0.001
Sex, <i>n</i> (%)			0.20
Male	32 (65.31)	38 (53.52)	
Female	17 (34.7)	33 (46.48)	
Age, years, mean \pm SD	51.4 \pm 14.2	47.8 \pm 17.8	0.22
BMI, kg/m ² , mean \pm SD	30.8 \pm 6.2	29.1 \pm 6.1	0.24
Psoriatic arthritis, <i>n</i> (%)	3 (6.12)	6 (8.45)	0.63

BSA: body surface area; DLQI: Dermatology Quality of Life Index; SD: standard deviation; BMI: body mass index.

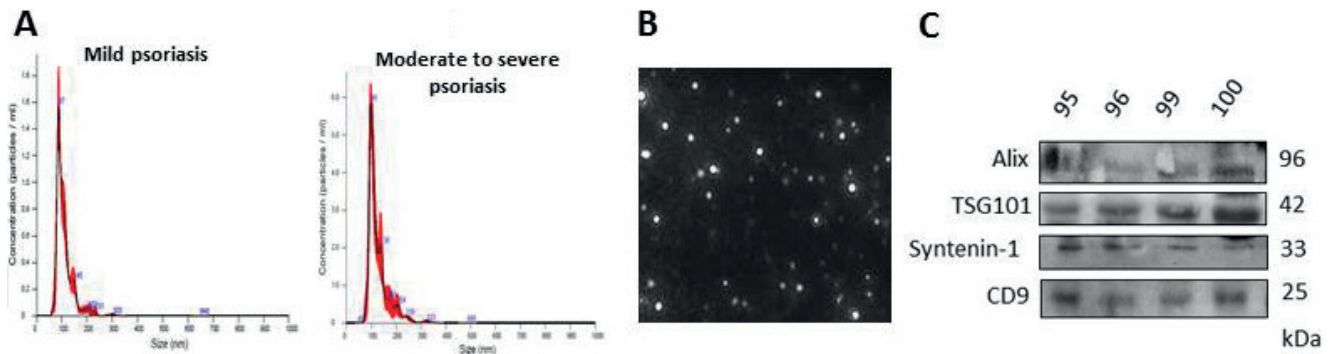


Fig. 1. (A) Representative distribution profile of exosomes isolated from patients with mild psoriasis and moderate-to-severe psoriasis by nanoparticles tracking analysis. (B) Representative image of exosomes derived from the plasma of a patient with psoriasis by nanoparticles tracking analysis. (C) Representative immunoblots expression of Alix, TSG101, Syntenin-1, CD9 in exosomes derived from the plasma of psoriasis patients (95 and 96: moderate-to-severe psoriasis; 99 and 100: mild psoriasis).

quantified (number and size) in only 58 psoriatic patients (33 patients with mild psoriasis and 25 patients with moderate-to-severe psoriasis) (**Fig. 1**).

In the psoriatic patients group, there was no significant difference regarding the number and size of exosomes, depending on psoriasis severity. In the mild psoriasis group, the mean exosome number was $2.52 \times 10^{11} \pm 2.29 \times 10^{10}$ and their mean size was 94.44 ± 22.00 nm; in the moderate-to-severe psoriasis group, the mean exosome number was $1.79 \times 10^{11} \pm 1.93 \times 10^{10}$, and their mean size was 96.87 ± 28.30 nm ($p=0.19$ and $p=0.72$, respectively, for exosome number and size).

Analysis of HSP70 in the exosomes

Intra-exosomal HSP70 was observed by western blotting and quantified for 22 patients with mild psoriasis and 16 patients with moderate-to-severe psoriasis, with no significant difference between the 2 groups (mean HSP70 0.44 ± 0.79 ng/ml in the mild psoriasis group vs. 0.85 ± 1.75 ng/ml in the moderate-to-severe psoriasis group ($p=0.48$)) (**Fig. 2**).

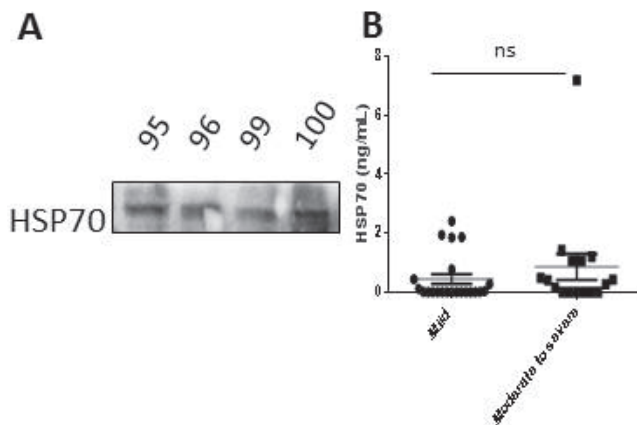


Fig. 2. (A) Representative immunoblot expression of intracellular of HSP70 in exosomes derived from the plasma of patients with psoriasis (95 and 96: moderate-to-severe psoriasis; 99 and 100: mild psoriasis). (B) Levels of HSP70 in the exosomes derived from plasma of psoriatic patients determined by enzyme-linked immunoassay (ELISA) (ns: not significant).

Exosomes and cytokines

Exosomal cytokines were quantified in 81 psoriatic patients (28 mild psoriasis patients and 53 patients with moderate-to-severe psoriasis). IL-17A exosome levels were significantly higher in patients with moderate-to-severe psoriasis compared with patients with mild psoriasis (2.3 pg/ml vs. 11.8 pg/ml, $p=0.02$). Among the 22 patients treated with IL-17 blockers, IL-17A exosome levels were also significantly higher in patients with moderate-to-severe psoriasis compared with patients with mild psoriasis ($p=0.02$). Exosomal TNF- α levels, IL-1- β , IL-2, IL-6 and IL-10 levels were similar in both groups of patients (Table II). Furthermore, no significant differences were observed between IL-17A exosome levels in patients treated with anti-IL-17 ($n=22$) compared with patients treated with other systemics, as in patients treated with the 2 anti-IL-17 blockers (Table II).

DISCUSSION

This study shows, for the first time, the presence of circulating exosomes in patients with psoriasis. Exosomes are involved in inflammatory processes, and their involvement in psoriasis has been suggested (6, 7). A previous study (12) demonstrated phospholipase A2 activity within IFN- α -induced mast cell-derived exosomes, which could be transferred to CD1a-expressing target cells. This led to the generation of neolipid antigens and subsequent recognition by lipid-specific CD1a-reactive

Table II. Cytokine released by exosomes

Cytokine level (pg/ml)	Mild psoriasis ($n=28$) Mean \pm SD	Moderate-to-severe psoriasis ($n=53$) Mean \pm SD	p -value
Exo-IL-17	2.3 \pm 5.4	11.8 \pm 5.1	0.02
Exo-IL-6	2.8 \pm 1	3.6 \pm 4.3	0.22
Exo-TNF- α	5 \pm 9.8	3.9 \pm 4.7	0.30
Exo-IL-1	4.2 \pm 4.2	4 \pm 2.4	0.77
Exo-IL-2	2.5 \pm 1.4	3 \pm 1.6	0.31
Exo-IL-10	3 \pm 1	3.2 \pm 1.3	0.66

SD: standard deviation. Significant value is shown in bold.

T cells inducing production of IL-22 and IL-17A. Altogether, these findings suggested that exosomes can transport potential ligands to neighbouring antigen-presenting dendritic cells involved in the early development of psoriasis. More recently, it has also been shown, in generalized pustular psoriasis, which is a severe and multisystemic form of psoriasis, that neutrophil exosomes amplify autoinflammatory responses in keratinocytes by up-regulating critical cytokines (IL-1 β , IL-18, IL-36, and TNF- α) and chemokines (CXCL1, CXCL2, CXCL8, and CCL20) for neutrophil infiltration (13).

We did not observe any significant differences between the number and size of exosomes from healthy patients and patients with psoriasis whatever the severity of psoriasis. These data illustrate that all cell types are able to release exosomes under physiological and pathological conditions.

Although emerging evidence has shown that stress stimuli provoke an increased release of exosome secretion (14), we did not find an increase in exosome release in patients with moderate-to-severe psoriasis compared with those with mild psoriasis. Furthermore, the number of HSP70 exosomes was similar in patients with mild psoriasis and in those with moderate-to-severe psoriasis. We do not have a clear explanation for this result, since psoriasis is considered an abnormal inflammatory and immune response to various pathogenic and environmental danger signals (8). The 70-kDa heat shock proteins, both constitutive and inducible forms, are known to be potent immunomodulators, with antagonistic activities, either promoting or down-regulating immune responses, depending on the context in which they appear in the extracellular space (15). The regulatory interplay between extracellular HSP70 and cytokine expression is also complicated.

In addition to transport of PLA2, this study showed that circulating exosomes from patients with psoriasis are able to transport pro-inflammatory cytokines (IL-1, IL-2, IL-6, IL-17A and TNF- α) and regulatory cytokines (IL-10). Furthermore, although we did not observe any significant difference between the number and size of exosomes and the severity of psoriasis, patients with moderate-to-severe psoriasis demonstrated higher IL-17A-exosome levels whatever the systemic treatment used, even with IL-17 blockers. These data thus suggest a key role of this cytokine in terms of response to treatment, since low levels of IL-17A-exosomes were associated with treatment-responsive psoriasis.

We do not have a clear explanation for the lack of association of other pro-inflammatory cytokines contained in exosomes with the severity of psoriasis. We suggest that these cytokines merely reflect the systemic inflammation whatever its cutaneous severity and the response to treatment.

Limitations of this study are the low patient numbers for subgroup analyses and detection mode of cytokines

by the assay. Serum measurements were not performed because results regarding serum levels of IL-17A are controversial. A recent meta-analysis concluded that levels of TNF- α , IFN- γ , IL-2, IL-6, IL-8, IL-18, IL-22 were higher in patients with psoriasis than in healthy controls, but this was not the case for IL-1 β , IL-4, IL-10, IL-12, IL-17A, IL-21, and IL-23 (16). Furthermore, there is some disagreement concerning the results of the correlation between the serum levels of IL-17A and psoriasis severity (17, 18). Differences in sample collection, storage and assessment methodologies may, in part, influence the conflicting results (16). In addition, a recent study demonstrated that exosomes provide a more consistent source of RNA than the whole plasma (19). The membrane of exosomes may protect the cargo from degradation in the bloodstream, and the intraluminal content is thought to be relatively stable.

In conclusion, these data confirm the involvement of circulating exosomes in psoriasis, in particular in terms of cutaneous severity, through IL-17A-producing exosomes. Further, larger studies are required to evaluate the effect of systemic treatments on exosome production. In addition, IL-17A exosomal components might represent a promising biomarker for assessing the therapeutic response to systemic treatment in patients with psoriasis.

ACKNOWLEDGEMENTS

The authors thank Elisabeth Homassel for her technical assistance.

This work was supported in part by the association A Fleur de Peau (Besançon, France).

Conflicts of interest: FA has served as a consultant and/or paid speaker for, and/or has received honoraria for consulting and/or scientific lectures for, and/or has received travel expenses reimbursed by, and/or has participated in clinical trials sponsored by companies that manufacture drugs used for the treatment of psoriasis, including AbbVie, Amgen, Boehringer Ingelheim, Celgene, Eli Lilly, Janssen-Cilag, LEO, MSD, Novartis, and Pfizer. The other authors have no conflicts of interest to declare.

REFERENCES

1. Théry C, Zitvogel L, Amigorena S. Exosomes: composition, biogenesis and function. *Nat Rev Immunol* 2002; 2: 569–579.
2. Février B, Raposo G. Exosomes: endosomal-derived vesicles shipping extracellular messages. *Curr Opin Cell Biol* 2004; 16: 415–421.
3. Cai J, Wu G, Jose PA, Zeng C. Functional transferred DNA within extracellular vesicles. *Exp Cell Res* 2016; 349: 179–183.
4. Daly M, O'Driscoll L. MicroRNA profiling of exosomes. *Methods Mol Biol* 2017; 1509: 37–46.
5. Skog J, Würdinger T, van Rijn S, Meijer DH, Gainche L, Sena-Esteves M, et al. Glioblastoma microvesicles transport RNA and proteins that promote tumour growth and provide diagnostic biomarkers. *Nat Cell Biol* 2008; 10: 1470–1476.
6. Console L, Scalise M, Indiveri C. Exosomes in inflammation and role as biomarkers. *Clin Chim Acta* 2018; 488: 165–171.
7. Wang WM, Wu C, Jin HZ. Exosomes in chronic inflammatory skin diseases and skin tumors. *Exp Dermatol* 2019; 28: 213–218.
8. Greb JE, Goldminz AM, Elder JT, Lebwohl MG, Gladman DD,

- Wu JJ, et al. Psoriasis. *Nat Rev Dis Primers* 2016; 2: 16082.
9. Seifarth FG, Lax JE, Harvey J, DiCorleto PE, Husni ME, Chandrasekharan UM, Tytell M. Topical heat shock protein 70 prevents imiquimod-induced psoriasis-like inflammation in mice. *Cell Stress Chaperones* 2018; 23: 1129–1135.
 10. Zhang B, Yin Y, Lai RC, Lim SK. Immunotherapeutic potential of extracellular vesicles. *Front Immunol* 2014; 5: 518.
 11. Gobbo J, Marcion G, Cordonnier M, Dias AMM, Pernet N, Hammann A, et al. Restoring anticancer immune response by targeting tumor-derived exosomes with a HSP70 peptide aptamer. *J Natl Cancer Inst* 2015; 108: 3.
 12. Cheung KL, Jarrett R, Subramaniam S, Salimi M, Gutowska-Owsiak D, Chen YL, et al. Psoriatic T cells recognize neolipid antigens generated by mast cell phospholipase delivered by exosomes and presented by CD1a. *J Exp Med* 2016; 213: 2399–2412.
 13. Shao S, Fang H, Zhang J, Jiang M, Xue K, Ma J, et al. Neutrophil exosomes enhance the skin autoinflammation in generalized pustular psoriasis via activating keratinocytes. *FASEB J* 2019; 33: 6813–6828.
 14. Vulpis E, Soriani A, Cerboni C, Santoni A, Zingoni A. Cancer exosomes as conveyors of stress-induced molecules: new players in the modulation of NK cell response. *Int J Mol Sci* 2019; 20: pii: E611.
 15. Calderwood SK, Gong J, Murshid A. Extracellular HSPs: the complicated roles of extracellular HSPs in immunity. *Front Immunol* 2016; 7: 159.
 16. Bai F, Zheng W, Dong Y, Wang J, Garstka MA, Li R et al. Serum levels of adipokines and cytokines in psoriasis patients: a systematic review and meta-analysis. *Oncotarget* 2018; 9: 1266–1278.
 17. El-Moaty Zaher HA, El-Komy MHM, Hegazy RA, Mohamed El Khashab HA, Ahmed HH. Assessment of interleukin-17 and vitamin D serum levels in psoriatic patients. *J Am Acad Dermatol* 2013; 69: 840–842.
 18. Kyriakou A, Patsatsi A, Vyzantiadis TA, Sotiriadis D. Serum levels of TNF- α , IL-12/23p40, and IL-17 in plaque psoriasis and their correlation with disease severity. *J Immunol Res* 2014; 467541.
 19. Endzeliņš E, Berger A, Melne V, Bajo-Santos C, Soboļevska K, Ābols A, et al. Detection of circulating miRNAs: comparative analysis of extracellular vesicle-incorporated miRNAs and cell-free miRNAs in whole plasma of prostate cancer patients. *BMC Cancer* 2017; 17: 730.